

# Package ‘MACPET’

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**Type** Package

**Title** Model based analysis for paired-end data

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**Description** The MACPET package can be used for complete interaction analysis for ChIA-PET data. MACPET reads ChIA-PET data in BAM or SAM format and separates the data into Self-ligated, Intra- and Inter-chromosomal PETs. Furthermore, MACPET breaks the genome into regions and applies 2D mixture models for identifying candidate peaks/binding sites using skewed generalized students-t distributions (SGT). It then uses a local poisson model for finding significant binding sites. Finally it runs an additive interaction-analysis model for calling for significant interactions between those peaks. MACPET is mainly written in C++, and it also supports the BiocParallel package.

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**Imports** intervals (>= 0.15.1), plyr (>= 1.8.4), Rsamtools (>= 2.1.3), stats (>= 3.6.1), utils (>= 3.6.1), methods (>= 3.6.1), GenomicRanges (>= 1.37.14), S4Vectors (>= 0.23.17), IRanges (>= 2.19.10), GenomeInfoDb (>= 1.21.1), gtools (>= 3.8.1), GenomicAlignments (>= 1.21.4), knitr (>= 1.23), rtracklayer (>= 1.45.1), BiocParallel (>= 1.19.0), Rbowtie (>= 1.25.0), GEOquery (>= 2.53.0), Biostrings (>= 2.53.2), ShortRead (>= 1.43.0), futile.logger (>= 1.4.3)

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**VignetteBuilder** knitr

**LinkingTo** Rcpp, bigmemory, BH

**SystemRequirements** C++11

**Collate** 'ALLData.R' 'AllClasses.R' 'AnalysisStatistics.R'  
'ConvertToPE\_BAM.R' 'ConvertToPSelf-methods.R'  
'GetShortestPath-methods.R' 'GetSignInteractions-methods.R'  
'InputChecks.R' 'MACPETUlt.R' 'MACPET\_pkg.R'  
'PeaksToGRanges-methods.R' 'PeaksToNarrowPeak-methods.R'  
'RcppExports.R' 'Stage\_0\_FilteringLinkersFunctions.R'  
'Stage\_1\_MappingFunctions.R'  
'Stage\_2\_PETClassificationFunctions.R'  
'Stage\_3\_PeakFinderFunctions.R'  
'Stage\_4\_InteractionsFunctions.R'  
'TagsToGInteractions-methods.R' 'exportPeaks-methods.R'  
'plot-methods.R' 'summary-methods.R'

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AnalysisStatistics      *Count Statistics for ChIA-PET.*

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## Description

AnalysisStatistics prints and saves count statistics for the current inputs of the peak-calling analysis.

## Usage

```
AnalysisStatistics(x.self, x.intra = NULL, x.inter = NULL,
  file.out = NULL, threshold = 1e-05, savedir = NULL)
```

## Arguments

x.self	An object of class <code>PSelf</code> or <code>PSFit</code> .
x.intra	An object of class <code>PIntra</code> (optional).
x.inter	An object of class <code>PInter</code> (optional).
file.out	A string with the name of the output to be saved to <code>savedir</code> . If <code>NULL</code> the function will only print the output.
threshold	A numeric indicating the FDR cut-off, used when <code>class(x.self)=PSFit</code> . If <code>NULL</code> , no threshold is applied.
savedir	A string with the directory to save the output file. If <code>NULL</code> then the function will only print the output.

## Value

Based on the inputs, AnalysisStatistics prints the total Self-ligated, Intra- and Inter-chromosomal PETs, as well as the total regions, total candidate peaks and total significant peaks (if `threshold!=NULL` and `class(x.self)=PSFit`). If `file.out` and `savedir` are not `NULL` then it also saves the output to a csv file in `savedir`.

## Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

## References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

## See Also

[PSelf](#), [PSFit](#), [PIntra](#), [PInter](#)

## Examples

```
#Create a temporary test folder, or anywhere you want:
savedir=file.path(tempdir(),'MACPETtest')
dir.create(savedir)#where you will save the results

#load Inter-chromosomal data:
load(system.file('extdata', 'MACPET_pinterData.rda', package = 'MACPET'))
class(MACPET_pinterData)

#load Intra-chromosomal data:
load(system.file('extdata', 'MACPET_pintraData.rda', package = 'MACPET'))
class(MACPET_pintraData)

#load Self-ligated data: (class=PSelf)
load(system.file('extdata', 'MACPET_pselfData.rda', package = 'MACPET'))
class(MACPET_pselfData)

#Print analysis:
AnalysisStatistics(x.self=MACPET_pselfData,
                  x.intra=MACPET_pintraData,
                  x.inter=MACPET_pinterData,
                  file.out='AnalysisStats',
                  savedir=savedir)

#####
#load Self-ligated data: (class=PSFit)
load(system.file('extdata', 'MACPET_psfiteData.rda', package = 'MACPET'))
class(MACPET_psfiteData)

#Print analysis:
AnalysisStatistics(x.self=MACPET_psfiteData,
                  x.intra=MACPET_pintraData,
                  x.inter=MACPET_pinterData,
                  file.out='AnalysisStats',
                  savedir=savedir,
                  threshold=1e-5)

#----delete test directory:
unlink(savedir,recursive=TRUE)
```

---

ConvertToPE_BAM	<i>Convert two BAM files into one paired-end BAM file.</i>
-----------------	--

---

### Description

Stage 2 in [MACPETU1t](#) needs a paired-end BAM file to run. This can be created in Stage 1 using the `usable_1` and `usable_2` fastq files created in Stage 0. However the user might have two single-end BAM files already created but not paired (by filtering with another way than that in Stage 0 or mapping using another algorithm than that in Stage 1) and only needs to run Stages 2 and 3 in [MACPETU1t](#). `ConvertToPE_BAM` can be used on the two BAM files for pairing them, and the resulted paired-end BAM file can then be used in Stage 2 in [MACPETU1t](#).

### Usage

```
ConvertToPE_BAM(S1_AnalysisDir = "", SA_prefix = "MACPET",  
S1_BAMStream = 2e+06, S1_image = TRUE, S1_genome = "hg19",  
BAM_file_1 = "", BAM_file_2 = "", S1_makeSam = FALSE)
```

### Arguments

<code>S1_AnalysisDir</code>	The directory where the resulted paired-end BAM file will be saved.
<code>SA_prefix</code>	see <a href="#">MACPETU1t</a> .
<code>S1_BAMStream</code>	see <a href="#">MACPETU1t</a> .
<code>S1_image</code>	see <a href="#">MACPETU1t</a> .
<code>S1_genome</code>	see <a href="#">MACPETU1t</a> .
<code>BAM_file_1</code>	The directory of the BAM file with the first reads. Their Qnames have to end with /1.
<code>BAM_file_2</code>	The directory of the BAM file with the second reads. Their Qnames have to end with /2.
<code>S1_makeSam</code>	see <a href="#">MACPETU1t</a> .

### Details

The BAM files `BAM_file_1` and `BAM_file_2` do not need to be sorted, but their Qnames have to end with /1 and /2 respectively. Furthermore, the BAM files have to include the header section.

### Value

A paired-end BAM file named `SA_prefix_MACPET_Paired_end.bam` and its index, saved in `S1_AnalysisDir`.

### Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

## References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

## See Also

[MACPETUlt](#), [SampleChIAPETDataRead\\_1.bam](#), [SampleChIAPETDataRead\\_2.bam](#)

## Examples

```
requireNamespace('ggplot2')

#Create a temporary folder, or anywhere you want:
S1_AnalysisDir=file.path(tempdir(),'MACPETtest')
dir.create(S1_AnalysisDir)#where you will save the results

#directories of the BAM files:
BAM_file_1=system.file('extdata', 'SampleChIAPETDataRead_1.bam', package = 'MACPET')
BAM_file_2=system.file('extdata', 'SampleChIAPETDataRead_2.bam', package = 'MACPET')
SA_prefix='MACPET'

#convert to paired-end BAM:
ConvertToPE_BAM(S1_AnalysisDir=S1_AnalysisDir,
                SA_prefix=SA_prefix,
                S1_BAMStream=2000000,
                S1_image=TRUE,
                S1_genome='hg19',
                BAM_file_1=BAM_file_1,
                BAM_file_2=BAM_file_2)

#test if the resulted BAM is paired-end:
PairedBAM=file.path(S1_AnalysisDir,paste(SA_prefix,'_Paired_end.bam',sep=''))
Rsamtools::testPairedEndBam(file = PairedBAM, index = PairedBAM)

bamfile = Rsamtools::BamFile(file = PairedBAM,asMates = TRUE)
GenomicAlignments::readGAlignmentPairs(file = bamfile,use.names = FALSE,
                                       with.which_label = FALSE,
                                       strandMode = 1)

#----delete test directory:
unlink(S1_AnalysisDir,recursive=TRUE)
```

## Description

ConvertToPSelf converts a [GInteractions](#) object to class to [PSelf](#) object.

## Usage

```
ConvertToPSelf(object, ...)  
  
## Default S3 method:  
ConvertToPSelf(object, ...)  
  
## S3 method for class 'GInteractions'  
ConvertToPSelf(object, S2_BlackList, SA_prefix,  
  S2_AnalysisDir, ...)  
  
## S4 method for signature 'GInteractions'  
ConvertToPSelf(object, S2_BlackList, SA_prefix,  
  S2_AnalysisDir, ...)
```

## Arguments

object	An object of <a href="#">GInteractions</a> class.
...	not used.
S2_BlackList	See <a href="#">MACPETUlt</a> .
SA_prefix	See <a href="#">MACPETUlt</a> .
S2_AnalysisDir	The directory in which the object will be saved.

## Details

[MACPETUlt](#) at State 2 separates the Inter-chromosomal, Intra-chromosomal and Self-ligated PETs by taking the paired-end BAM/SAM file as input. However the user might only have Self-ligated data available and already separated from the Inter/Intra-chromosomal PETs. [ConvertToPSelf](#) can then be used in the Self-ligated data to convert a [GInteractions](#) object containing only the Self-ligated PETs to a [PSelf](#) class for further analysis in Stage 3. The object will be saved in the `S2_AnalysisDir` directory with the name `SA_prefix_pselfData`. Note that if `S2_BlackList==TRUE` then the [GInteractions](#) object given as input has to include the genome name in the `seqinfo` slot. Also, the sequences lengths are mandatory in the `seqinfo` slot since they are used in stage 3 of the analysis.

## Value

An object of class [PSelf](#).

## Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

## References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

## See Also

[PSelf](#)

## Examples

```
#load Self-ligated data: (class=PSelf)
load(system.file('extdata', 'MACPET_pselfData.rda', package = 'MACPET'))
class(MACPET_pselfData)

object=MACPET_pselfData
#--remove information and convert to GInteractions:
S4Vectors::metadata(object)=list(NULL)
class(object)='GInteractions'
#----input parameters
S2_BlackList=TRUE
SA_prefix='MACPET'
S2_AnalysisDir=file.path(tempdir(),'MACPETtest')
if(!dir.exists(S2_AnalysisDir)) dir.create(S2_AnalysisDir)

ConvertToPSelf(object=object,
               S2_BlackList=S2_BlackList,
               SA_prefix=SA_prefix,
               S2_AnalysisDir=S2_AnalysisDir)

#load object:
rm(MACPET_pselfData)#old object
load(file.path(S2_AnalysisDir,'MACPET_pselfData'))
class(MACPET_pselfData)
#-----delete test directory:
unlink(S2_AnalysisDir,recursive=TRUE)
```

---

exportPeaks

*Exports peaks to csv file*

---

## Description

exportPeaks is an S3 method for the [PSFit](#) class. It exports peak information to a csv file in a given directory.

## Usage

```
exportPeaks(object, ...)
```

```
## Default S3 method:
```



```
exportPeaks(object, ...)  
  
## S3 method for class 'PSFit'  
exportPeaks(object, file.out, savedir, threshold = NULL,  
  ...)  
  
## S4 method for signature 'PSFit'  
exportPeaks(object, file.out, savedir,  
  threshold = NULL, ...)
```

### Arguments

object	An object of <a href="#">PSFit</a> class.
...	(not used).
file.out	A string with the name of the output to be saved to savedir.
savedir	A string with the directory to save the output.
threshold	A numeric indicating the FDR cut-off used for subsetting significant peaks. If NULL all the peaks are returned.

### Value

For [PSFit](#) class: a csv file named after the value of `file.out` with all the information about the peaks found by the [MACPETUlt](#) function, plus comments which explain the column names.

### Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

### References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

### See Also

[PSFit](#)

### Examples

```
#Create a temporary folder, or anywhere you want:  
savedir=file.path(tempdir(), 'MACPETtest')  
dir.create(savedir)#where you will save the results  
  
#load Self-ligated data: (class=PSFit)  
load(system.file('extdata', 'MACPET_psfidData.rda', package = 'MACPET'))  
class(MACPET_psfidData)  
exportPeaks(object=MACPET_psfidData, file.out='Peaks', threshold=1e-5, savedir=savedir)  
  
#-----delete test directory:  
unlink(savedir, recursive=TRUE)
```

---

GenomeMap-class	<i>GenomeMap S4 Class</i>
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---

## Description

[GenomeMap](#) class is a S4 class which inherits from the [GInteractions](#) class and it contains all the interactions found by MACPET at Stage 4. This class represents the genome map constructed by the peaks which interact. Furthermore, in the [metadata](#) field, it contains a [DataFrame](#) called [InteractionInfo](#) with the interaction information. More precisely, the [InteractionInfo](#) object contains the following columns:

[pvalue](#) The p-value of the interaction.

[FDR](#) The FDR of the interaction.

[Order](#) The order the interaction was entered into the model.

[TotalInterPETs](#) The total interaction PETs between every two interacting peaks.

Finally it also contains two metadata columns with the peak summits of the two peaks in each interaction.

## Details

[GenomeMap](#) class is created by the [MACPETUlt](#) function at Stage 4. Interactions with same [Order](#) are those who entered the model together because they had the same [FDR](#) value. Subsets of significant interactions can be found using the [GetSignInteractions](#) function.

## Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

## References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Complete pipeline for ChIA-PET*. To be published.

## See Also

[plot](#), [summary](#), [GetSignInteractions](#), [MACPETUlt](#), [GetShortestPath](#)

---

GetShortestPath	<i>Find shortest path between pairs of peaks given a set of significant interactions.</i>
-----------------	---

---

### Description

GetShortestPath function subsets the significant interactions of a [GenomeMap](#) object given a user specified FDR cut-off. Based on the leftover interactions, it creates a network and finds the length of the shortest path between two user-specified peaks. Currently it only finds the shortest paths between intra-chromosomal peaks. Therefore, the peaks have to be on the same chromosome.

### Usage

```
GetShortestPath(object, ...)  
  
## Default S3 method:  
GetShortestPath(object, ...)  
  
## S3 method for class 'GenomeMap'  
GetShortestPath(object, threshold = NULL, ChrFrom,  
  ChrTo, SummitFrom, SummitTo, ...)  
  
## S4 method for signature 'GenomeMap'  
GetShortestPath(object, threshold = NULL, ChrFrom,  
  ChrTo, SummitFrom, SummitTo, ...)
```

### Arguments

object	An object of class <a href="#">GenomeMap</a> .
...	Further arguments to be passed to GetShortestPath (not used).
threshold	A numeric with the FDR cut-off threshold used to take a subset of significant interactions. If threshold=NULL then all the interactions will be used.
ChrFrom	Character specifying the chromosome of the 'From' peak, for example 'chr1'.
ChrTo	Character specifying the chromosome of the 'To' peak.
SummitFrom	Numeric specifying the peak summit of the 'From' peak.
SummitTo	Numeric specifying the peak summit of the 'To' peak.

### Value

A two-element list with the first element named `LinearPathLength` for the linear length of the path between `SummitFrom` and `SummitTo`, and the second element named `ThreeDPathLength` for the 3D length of the shortest path between `SummitFrom` and `SummitTo`.

### Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

## References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Complete pipeline for ChIA-PET*. To be published.

## See Also

[GenomeMap](#)

## Examples

```
#load Interaction data: (class=GenomeMap)
load(system.file('extdata', 'MACPET_GenomeMapData.rda', package = 'MACPET'))
class(MACPET_GenomeMapData)
GetShortestPath(object=MACPET_GenomeMapData,
                threshold = NULL,
                ChrFrom='chr1',
                ChrTo='chr1',
                SummitFrom=10000,
                SummitTo=1000000)
```

---

GetSignInteractions    *Get the significant interactions of a [GenomeMap](#) object*

---

## Description

GetSignInteractions subsets the significant interactions of a [GenomeMap](#) object given a user specified FDR cut-off. It returns/saves different kind of objects based on the ReturnedAs value.

## Usage

```
GetSignInteractions(object, ...)

## Default S3 method:
GetSignInteractions(object, ...)

## S3 method for class 'GenomeMap'
GetSignInteractions(object, threshold = NULL,
                    ReturnedAs = "GInteractions", SaveTo = "", Prefix = "MACPET", ...)

## S4 method for signature 'GenomeMap'
GetSignInteractions(object, threshold = NULL,
                    ReturnedAs = "GInteractions", SaveTo = "", Prefix = "MACPET", ...)
```

**Arguments**

object	An object of class <a href="#">GenomeMap</a> .
...	Further arguments to be passed to GetSignInteractions (not used).
threshold	A numeric with the FDR cut-off threshold used to take a subset of significant interactions. If threshold=NULL then all the interactions are returned.
ReturnedAs	What type of object to return. Possible values are: 'GInteractions' Will return a <a href="#">GInteractions</a> object. Each line corresponds to an interaction between two anchors (peaks). It provides information about the intervals of the peaks, the anchor/peak summits, the interaction p-value/FDR and order (see <a href="#">GenomeMap</a> for further details). 'csv' Will save a .csv file in the SaveTo. The file will be named after the Prefix parameter Prefix_InteractionResults.csv
SaveTo	The directory to save the csv file in case of ReturnedAs=='csv'.
Prefix	A string which will be used a prefix on the object saved in SaveTo.

**Details**

[MACPETUI](#) at Stage 4 will run the interaction analysis for the ChIA-PET data. This stage will process all the interactions in the data. After Stage 4 is run, the user can use the GetSignInteractions function on the saved object of class [GenomeMap](#), to subset the significant interactions given a FDR cut-off.

**Value**

For [GenomeMap](#) class, different outputs based on the ReturnedAs parameter.

**Author(s)**

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**References**

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Complete pipeline for ChIA-PET*. To be published.

**See Also**

[GenomeMap](#)

**Examples**

```
#load Interaction data: (class=GenomeMap)
load(system.file('extdata', 'MACPET_GenomeMapData.rda', package = 'MACPET'))
class(MACPET_GenomeMapData)
GetSignInteractions(object=MACPET_GenomeMapData,
                    threshold = NULL,
                    ReturnedAs='GInteractions')
```

## Description

The MACPET package can be used for general analysis of paired-end (PET) data like ChIA-PET. MACPET currently implements the following five stages: Linker filtering (stage 0), mapping to the reference genome (stage 1), PET classification (stage 2), peak-calling (stage 3) and interaction analysis (stage 4). The interaction analysis stage (4) currently supports only intra-chromosomal data. All of the MACPET stages can be run at once, or separately. In stage 0, MACPET identifies the linkers in the fastq files and classifies the reads as usable, chimeric or ambiguous. Usable reads are considered in the subsequent stages. In stage 1, MACPET maps the usable reads to the reference genome using [bowtie](#) and produces a paired-end BAM file. This BAM file is further used in stage 2 to classify the PETs as self-ligated/intra- or inter-chromosomal. Self-ligated PETs are used in stage 3 for the identification of significant peaks. In stage 3, MACPET segments the genome into regions and applies 2D mixture models for identifying candidate peaks using skewed generalized students-t distributions (SGT). It then uses a local poisson model for finding significant binding sites. Finally, in stage 4, MACPET uses the peaks from stage 3 as well as the Intra-chromosomal PETs from stage 2 to running an additive interaction-analysis model. MACPET is mainly written in C++, and it supports the BiocParallel package.

## MACPET main function

[MACPETUlt](#) runs the whole analysis at once.

## MACPET classes

[PSelf](#) S4 class for Self-ligated PETs.

[PSFit](#) S4 class for Self-ligated PETs after peak-calling.

[PInter](#) S4 class for Inter-chromosomal PETs.

[PIntra](#) S4 class for Intra-chromosomal PETs.

[GenomeMap](#) S4 class for the interactions found in the data.

## MACPET methods

[plot](#) Method for plotting different objects.

[summary](#) Method for summarizing different objects.

[TagsToGInteractions](#) Method for converting Tags to [GInteractions](#) class.

[PeaksToGRanges](#) Method for converting peaks to [GRanges](#) class.

[exportPeaks](#) Method for exporting peaks in cvs file format.

[ConvertToPSelf](#) Method for converting a [GInteractions](#) class of Self-ligated PETs to object of [PSelf](#) class.

[GetSignInteractions](#) Method for subsetting the significant interactions of a [GenomeMap](#) object.

[GetShortestPath](#) Method for finding the shortest path between two peaks given a set of interactions.

[PeaksToNarrowPeak](#) Method for converting peaks to narrowPeak (BED) format for use in interaction analysis using the MANGO/MICC algorithm. Alternatively the user can run stage 4 at [MACPETU1t](#).

### MACPET supplementary functions

[ConvertToPE\\_BAM](#) Function for converting two separate BAM files into one paired-end BAM file.

[AnalysisStatistics](#) Prints summary of multiple objects.

### MACPET sample data

[SampleChIAPETData.bam](#) Sample ChIA-PET data.

[SampleChIAPETDataRead\\_1.bam](#) First reads from the sample ChIA-PET data.

[SampleChIAPETDataRead\\_2.bam](#) Second reads from the sample ChIA-PET data.

[MACPET\\_pinterData.rda](#) Sample [PInter](#) data.

[MACPET\\_pintraData.rda](#) Sample [PIntra](#) data.

[MACPET\\_pselfData.rda](#) Sample [PSelf](#) data.

[MACPET\\_psfidData.rda](#) Sample [PSFit](#) data.

[MACPET\\_GenomeMapData.rda](#) Sample [GenomeMap](#) data.

### Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

### References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Complete pipeline for ChIA-PET*. To be published.

### Description

MACPETU1t is used for running analysis based on paired-end DNA data, including stages for linker removal, mapping to the reference genome, PET classification, binding site identification and interaction analysis.

**Usage**

```
MACPETUlt(SA_AnalysisDir = "", SA_stages = c(0:3),
  SA_prefix = "MACPET", S0_fastq1 = "", S0_fastq2 = "",
  S0_LinkerA = "GTTGGATAAG", S0_LinkerB = "GTTGGAATGT",
  S0_MinReadLength = 18, S0_MaxReadLength = 50,
  S0_LinkerOccurrence = 0, S0_image = TRUE, S0_fastqStream = 2e+06,
  S1_fastq1_usable_dir = "", S1_fastq2_usable_dir = "",
  S1_image = TRUE, S1_BAMStream = 2e+06, S1_makeSam = TRUE,
  S1_genome = "hg19", S1_RbowtieIndexBuild = FALSE,
  S1_RbowtieIndexDir = "", S1_RbowtieIndexPrefix = "",
  S1_RbowtieRefDir = "", S2_PairedEndBAMpath = "", S2_image = TRUE,
  S2_BlackList = TRUE, S3_fileSelfDir = "", S3_image = TRUE,
  S3_method = "BH", S4_filePSFitDir = "", S4_filePIntraDir = "",
  S4_filePInterDir = NULL, S4_FDR_peak = 0.1, S4_method = "BH",
  S4_image = TRUE, S4_minPETs = 2, S4_PeakExt = 500)
```

**Arguments**

- SA\_AnalysisDir** A directory where all the output is to be saved. This parameter is mandatory for every stage.
- SA\_stages** Numeric vector or integer (if stages are run separately). This parameter is mandatory for every stage (see details).
- SA\_prefix** A string which is going to be used as prefix for the outputs (default: 'MACPET'). This parameter is mandatory for every stage.
- S0\_fastq1** A string with the directory of the 5-end fastq (or fastq.gz) file. This parameter is mandatory if Stage 0 is run (see details).
- S0\_fastq2** A string with the directory of the 3-end fastq (or fastq.gz) file. This parameter is mandatory if Stage 0 is run (see details).
- S0\_LinkerA** A string with the first linker sequence (default 'GTTGGATAAG'). This parameter is mandatory if Stage 0 is run (see details).
- S0\_LinkerB** A string with the second linker sequence (default 'GTTGGAATGT'). This parameter is mandatory if Stage 0 is run (see details).
- S0\_MinReadLength** A positive integer with the minimum read length after linker trimming (default: 18). This parameter is mandatory if Stage 0 is run (see details).
- S0\_MaxReadLength** A positive integer with the maximum read length after linker trimming (default: 50). This parameter is mandatory if Stage 0 is run (see details).
- S0\_LinkerOccurrence** One of the following: 0, 1, 2, 3, 4. This parameter defines the linker-occurrence mode (see details). Default 0.
- S0\_image** Logical, indicating if a pie-chart image for the fastq files classification will be produced (default=TRUE). This parameter is mandatory if Stage 0 is run.
- S0\_fastqStream** Positive integer for total lines of fastq files to be loaded in R (best to leave it at default because it might cause memory crash). This parameter is mandatory if Stage 0 is run.



S1_fastq1_usable_dir	String with the directory of the 5-end usable fastq (or fastq.gz) files. This parameter might not be mandatory (see details).
S1_fastq2_usable_dir	String with the directory of the 3-end usable fastq (or fastq.gz) files. This parameter might not be mandatory (see details).
S1_image	Logical indicating if images for the mapping percentage and the pairing percentage will be produced (default=TRUE). This parameter is mandatory if Stage 1 is run.
S1_BAMStream	Positive integer for the total number of bam file lines to be loaded in R in a loop for pairing (best to leave it at default because it might cause memory crash). This parameter is mandatory if Stage 1 is run.
S1_makeSam	Logical indicating whether the resulted paired-end BAM file will be splitted to two SAM files (one for each read). The output SAM files can be used as input in the MANGO algorithm (default=TRUE). Note, that the user has to remove the SAM header before running MANGO. This parameter is mandatory if Stage 1 is run.
S1_genome	String with the genome to be used in the bam file header (default='hg19'). This parameter is mandatory if Stage 1 is run (see details).
S1_RbowtieIndexBuild	Logical indicating whether you want to build the bowtie index or not (default=FALSE). This parameter is mandatory if Stage 1 is run (see details).
S1_RbowtieIndexDir	String with the directory of the bowtie index (if S1_RbowtieIndexBuild==FALSE) or with the directory where the bowtie index will be saved (if S1_RbowtieIndexBuild==TRUE). This parameter is mandatory if Stage 1 is run (see details).
S1_RbowtieIndexPrefix	String with the prefix for the bowtie indeces in S1_RbowtieIndexDir (see details). This parameter is mandatory if Stage 1 is run (see details).
S1_RbowtieRefDir	A vector with the directories of the .fa files, used if S1_RbowtieIndexBuild==TRUE. This parameter is mandatory if Stage 1 is run and S1_RbowtieIndexBuild==TRUE (see details).
S2_PairedEndBAMpath	A string with the directory of the paired-end bam file (or paired-end sam file). This parameter might not be mandatory (see details).
S2_image	Logical indicating whether images for the self-ligated/intra-chromosomal cut-off as well as pie-charts for the PET classification will be produced (default=TRUE). This parameter is mandatory if Stage 1 is run.
S2_BlackList	Logical indicating whether black-listed regions will be removed from the data based on the S1_genome parameter (see details). Alternatively a GRanges object with the user specified regions. This parameter is mandatory if Stage 2 is run.
S3_fileSelfDir	A string with the directory of the of the object of class PSelf. This parameter might not be mandatory (see details).

S3_image	Logical indicating whether images for the binding site's FDR, sizes of the binding sites, sizes of binding site's upstream/downstream peaks will be created. This parameter is mandatory if Stage 3 is run.
S3_method	String with the FDR method used for finding p-values of significant peaks in the data. See <a href="#">p.adjust.methods</a> (default= 'BH'). This parameter is mandatory if Stage 3 is run.
S4_filePSFitDir	A string with the directory of the object of class <a href="#">PSFit</a> . This parameter is not mandatory if Stage 3 is run right before Stage 4.
S4_filePIntraDir	A string with the directory of the object of class <a href="#">PIntra</a> . This parameter is not mandatory if Stages 2:3 are run right before Stage 4.
S4_filePInterDir	A string with the directory of the object of class <a href="#">PInter</a> . This parameter is not mandatory if Stages 2:3 are run right before Stage 4. NOTE: Currently, inter-chromosomal interactions are not supported, so this parameter is ignored.
S4_FDR_peak	A numeric for the FDR cut-off value of significant peaks to be used in interaction analysis (default 0.1 because MACPET is more strict in peak-calling than MACS, higher threshold than 0.1 might also be appropriate). This parameter is mandatory if Stage 4 is run.
S4_method	String with the FDR method used for finding p-values of significant interactions in the data. See <a href="#">p.adjust.methods</a> (default= 'BH'). This parameter is mandatory if Stage 4 is run.
S4_image	Logical indicating whether images for the total peaks and total intra- and inter-chromosomal PETs used in the interaction analysis will be created. This parameter is mandatory if Stage 4 is run.
S4_minPETs	The minimum total PETs allowed between every two peaks (default=2). This parameter is mandatory if Stage 4 is run.
S4_PeakExt	Integer for the number of bp to extend each peak on either side (default 500). This parameter will both affect the interaction PETs falling into the peak and the merging of overlapping peaks. This parameter is mandatory if Stage 4 is run.

## Details

Every stage has parameters associated with it. Parameters with prefix SA correspond to all stages, S0 to Stage 0, S1 to Stage 1 etc. Parameters with SA prefix are mandatory for every stage.

If SA\_stages parameter is given as vector, then the vector has to be continuous, that is for example c(0:3) or c(2:3), not c(0,2,3). In general the best practice is to run all the stages at once.

The fastq files in S0\_fastq1 and S0\_fastq2 have to be of same length and be sorted by their ID. Furthermore, the IDs in S0\_fastq1 have to end with /1 and the ones in S0\_fastq2 with /2, representing the 5- and 3-end tags respectively. In other words, for the same line in S0\_fastq1 and S0\_fastq2, their IDs have to be identical, except from their suffixes /1 and /2 respectively. Moreover, the '/' symbol can be replaced with any other symbol, this will not cause any problems.

S0\_LinkerOccurrence parameter defines the linker-occurrence mode and separates the usable from the ambiguous PETs. PETs with both reads including linkers are not affected by S0\_LinkerOccurrence.

Also, reads which do not meet the `S0_MaxReadLength/S0_MinReadLength` lengths, are moved to ambiguous anyway. The four values of `S0_LinkerOccurrence` are:

- Mode 0: Both reads have to include a linker in order to be checked as usable or chimeric, if they dont, they are moved to ambiguous.
- Mode 1: If read 1 is not matching any linker, but read 2 does, then the PET will be moved to usable.
- Mode 2: If read 2 is not matching any linker, but read 1 does, then the PET will be moved to usable.
- Mode 3: If any of the reads does not match any linker then the PET they will be moved to usable.
- Mode 4: If both reads do not match any of the linkers, then the PET will be moved to usable.

`S0_MaxReadLength` has to be greater than `S0_MinReadLength`. The user should leave those two at default unless the PET data is produced by tagmentation.

`S1_fastq1_usable_dir` and `S1_fastq2_usable_dir` are not mandatory if Stage 0 is run right before Stage 1 (`SA_stages=c(0,1)`). Those two are only mandatory if Stage 1 is run separately. Then those parameters assume to have the usable reads only. The same fastq specifications apply as those for `S0_fastq1` and `S0_fastq2`.

The parameter `S1_genome` is very important. First the genome name given in `S1_genome` should be the same as the one used for building the bowtie index for mapping. This parameter will add an 'AS' column to the paired-end bam file with the genome information. In Stage 2, this header will be used for identifying which kind on black-listed regions to use if `S2_BlackList==TRUE`.

If `S1_RbowtieIndexBuild==FALSE` then the bowtie index is assumed to be already built and saved in `S1_RbowtieIndexDir`. Then the `S1_RbowtieIndexDir` folder should include the following files: `S1_RbowtieIndexPrefix.1.ebwt`, `S1_RbowtieIndexPrefix.2.ebwt`, `S1_RbowtieIndexPrefix.3.ebwt`, `S1_RbowtieIndexPrefix.4.ebwt`, `S1_RbowtieIndexPrefix.rev.1.ebwt` and `S1_RbowtieIndexPrefix.rev.2.ebwt`, or with `.ebwtl`. Where `S1_RbowtieIndexPrefix` is also given as input.

If `S1_RbowtieIndexBuild==TRUE` then the bowtie index will be build using the `bowtie_build` function. This function will need the `.fa` files which should be given as input in the `S1_RbowtieRefDir` vector. This is a character vector with the directories of the `.fa` files to use. The output index will be saved in `S1_RbowtieIndexDir`. if `S1_RbowtieIndexBuild==FALSE` then `S1_RbowtieRefDir` can be an empty string.

The parameter `S2_PairedEndBAMpath` has to be specified only if Stage 2 is run without running Stage 1 right before (`SA_Stages=c(2)` or `c(2,3)`, not `c(1,2)` or `c(0,1,2)` for example). If this is the case, the `S2_PairedEndBAMpath` has to be the path to the BAM/SAM paired-end file. The file has to include the header with the 'SN', 'LN' and 'AS' columns. Moreover the mate flags of the file have to be correct and also the duplicated PETs must be flagged too. Stage 2 will upload the whole data in R using `readGAlignmentPairs` function with flags `isDuplicate=FALSE` and `isPaired=TRUE`. So if duplicated PETs are not flagged, they will be used in the analysis. If the previous stages are run in sequence, then `S2_PairedEndBAMpath` will be overwritten with the newly created BAM file, which will have the correct flags.

If `S2_BlackList==TRUE` then which genome black-list is going to be used is decided by the 'AS' column in the `S2_PairedEndBAMpath` file, which is specified by the `S1_genome` if Stage 1 is also run. The black-listed regions cover the following genomes: 'hg19', 'ce10', 'dm3', 'hg38', 'mm9', 'mm10'. If the 'AS' header column is missing from the `S2_PairedEndBAMpath` file, or if the `S1_genome` is not matching any of the above named genomes, then a warning will be produced saying that no black-listed regions will be removed. Alternatily, the user can provide its own black-listed regions as a `GRanges` object.

The parameter `S3_fileSelfDir` is not mandatory if the stages are run in sequence, if Stage 2 is run right before stage 3. If this is the case then `S3_fileSelfDir` will be overwritten with the data produced in Stage 2. If Stage 3 is run separately, then `S3_fileSelfDir` has to be provided. It should be a `PSelf` object and both the name of the object in the directory and the one uploaded in R should be `SA_prefix_pselfData`.

## Value

All outputs are saved at the `SA_AnalysisDir`. The output depends on the stages run:

**Stage 0: (outputs saved in a folder named `S0_results` in `SA_AnalysisDir`)** `SA_prefix_usable_1.fastq.gz`: fastq.gz files with the usable 5-end tags. To be used in Stage 1.

`SA_prefix_usable_2.fastq.gz`: fastq.gz files with the usable 3-end tags. To be used in Stage 1.

`SA_prefix_chimeric_1.fastq.gz`: fastq.gz files with the chimeric 5-end tags.

`SA_prefix_chimeric_2.fastq.gz`: fastq.gz files with the chimeric 3-end tags.

`SA_prefix_ambiguous_1.fastq.gz`: fastq.gz files with the ambiguous 5-end tags.

`SA_prefix_ambiguous_2.fastq.gz`: fastq.gz files with the ambiguous 3-end tags.

`SA_prefix_stage_0_image.jpg`: Pie chart image with the split of two fastq files used as input (if `S0_image==TRUE`).

**Stage 1: (outputs saved in a folder named `S1_results` in `SA_AnalysisDir`)** `SA_prefix_usable_1.sam`: sam file with the mapped 5-end reads (if `S1_makeSam==FALSE`).

`SA_prefix_usable_2.sam`: sam file with the mapped 3-end reads (if `S1_makeSam==FALSE`).

`SA_prefix_Paired_end.bam`: paired-end bam file with the mapped PETs. To be used in Stage 2

`SA_prefix_Paired_end.bam.bai`: .bai file for `SA_prefix_Paired_end.bam`. To be used in Stage 2.

`SA_prefix_stage_1_p1_image.jpg`: Pie-chart for the mapped/unmapped reads from `SA_prefix_usable_1.sam`, `SA_prefix_usable_2.sam` (if `S1_image==TRUE`).

`SA_prefix_stage_1_p2_image.jpg`: Pie-chart for the paired/unpaired reads of `SA_prefix_Paired_end.bam` (if `S1_image==TRUE`).

**Stage 2: (outputs saved in a folder named `S2_results` in `SA_AnalysisDir`)** `SA_prefix_pselfData`: An object of `PSelf` class with the Self-ligated PETs. To be used in Stage 3.

`SA_prefix_pintraData`: An object of `PIntra` class with the Intra-chromosomal PETs.

`SA_prefix_pinterData`: An object of `PInter` class with the Inter-chromosomal PETs.

`SA_prefix_stage_2_p1_image.jpg`: Pie-chart reliable/duplicated/black-listed PETs of `SA_prefix_Paired_end.bam` (if `S2_image==TRUE`).

`SA_prefix_stage_2_p2_image.jpg`: Histogram with the self-ligated/intra-chromosomal cut-off for `SA_prefix_Paired_end.bam` (if `S2_image==TRUE`).

`SA_prefix_stage_2_p3_image.jpg`: Pie-chart for the self-ligated/intra-chromosomal/inter-chromosomal PETs of `SA_prefix_Paired_end.bam` (if `S2_image==TRUE`).

**Stage 3: (outputs saved in a folder named `S3_results` in `SA_AnalysisDir`)** `SA_prefix_psfitaData`: An object of `PSFit` class with the peak information.

`SA_prefix_stage_3_p1_image.jpg`: Sizes of the upstream vs downstream peaks of each binding site given the binding site's FDR (if `S3_image==TRUE`).

SA\_prefix\_stage\_3\_p2\_image.jpg: FDR of the binding sites. The horizontal red line is at FDR=0.05 (if S3\_image==TRUE).

SA\_prefix\_stage\_3\_p3\_image.jpg: Comparison of binding site sizes given their FDR (if S3\_image==TRUE).

SA\_prefix\_stage\_3\_p3\_image.jpg: FDR for the upstream/downstream peaks of the binding sites given the binding sites FDR (if S3\_image==TRUE).

**Stage 4: (outputs saved in a folder named S4\_results in SA\_AnalysisDir)** SA\_prefix\_GenomeMapData: An object of [GenomeMap](#) class with the interactions information.

SA\_prefix\_stage\_4\_p1\_image.jpg: Pie charts with the total peaks involved in the interactions and the total intra/inter-chromosomal PETs involved in the interactions (if S4\_image==TRUE).

**Stage 0:4 :** All the above outputs. Furthermore, a log file named SA\_prefix\_analysis.log is always created in SA\_AnalysisDir with information about the process.

### Stages description

MACPETUlt runs a complete or partial analysis for PET data, depending on the stages of the analysis the user wants to run. The stages of the analysis are the following:

**Stage 0:** Linker identification stage: This stage uses the two fastq files for the 5- and 3-end tags and identifies which tags contain any of the linkers. Based on the linker combinations it classifies the PETs as usable (linkers A/A or B/B), chimeric (linkers A/B or B/A) and ambiguous (linkers non/A, non/B, A/non, B/non unless chosen otherwise by S0\_LinkOccurrence, or be smaller/bigger than the S0\_MinReadLength/S0\_MaxReadLength after the linker removal, respectively). Only usable PETs are considered in the subsequent steps.

**Stage 1:** PET mapping stage: This stage uses the usable PETs identified by stage 0. It maps them separately to the reference genome using the [bowtie](#) function with no mismatch per read, and keeps the uniquely mapped reads only. It then maps the unmapped reads to the reference genome with at most one mismatch and keeps the uniquely mapped reads. Uniquely mapped reads with zero or one mismatch are then merged and paired, their duplicates are marked and a paired-end bam file is created which is used in State 2.

**Stage 2:** PET classification stage: This stage takes the BAM paired-end file from stage 1 and classifies the PETs as: Inter-chromosomal PETs (which connect two different chromosomes), Intra-chromosomal PETs (which connect regions of the same chromosome) and Self-ligated PETs (which are used for binding site analysis). Self-ligated PETs are used for finding the protein binding sites (peaks), while Intra- and Inter-chromosomal are used for interactions between the peaks. The algorithm uses the elbow-method to separate the Self-ligated from the Intra-chromosomal population. Note that loading the data into R might take a while depending on the size of the data.

**Stage 3:** Peak calling stage: This stage uses the Self-ligated PETs and it runs the EM algorithm to find clusters which represent candidate peaks/binding sites in 2 dimensional space using skewed generalized students-t distributions (SGT). After the peak-calling analysis is done, the algorithm assesses the significance of the candidate peaks using a local Poisson model.

**Stage 4:** Interaction analysis stage: This stage uses the peaks found in stage 3 and the intra-chromosomal PETs found in stage 2 to discover interactions between those peaks. Inter-chromosomal PETs are not supported yet. It first estimates the expected number of PETs in

each interaction under  $H_0$  (everything is random), based on the depth of the peaks which interact and their distance. It then uses the Poisson distribution to call for significant interactions. The resulting object will include all potential interactions found in the data. Then the function `GetSignInteractions` can be used for subsetting significant interactions. Therefore, this stage needs only to be run once, then the FDR cut-off of the most significant interactions can be given multiple times in the `GetSignInteractions` function. This stages will estimate the interactions in circles. In each circle the most significant interactions will be added to the model (those with the lowest FDR). Then the distances between the peaks of the significant interactions will become 0 and the rest of the distances (for the interactions not added yet) will be adjusted based on that. Then the second circle will run and so on.

### Parallel

All stages can be run in parallel using the `register` function. The user has to register a parallel backhead before starting the function.

### Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

### References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Complete pipeline for ChIA-PET*. To be published.

Consortium EP (2012) *An integrated encyclopedia of DNA elements in the human genome..* Nature, 489(7414), pp. 57–74. <http://dx.doi.org/10.1038/nature11247>.

### See Also

`PSelf`, `PIntra`, `PInter`, `PSFit`, `GenomeMap`, `summary`, `AnalysisStatistics`, `plot` `BiocParallel`, `ConvertToPSelf`, `exportPeaks`, `TagsToGInteractions`, `PeaksToGRanges`, `PeaksToNarrowPeak`, `ConvertToPE_BAM`, `GetSignInteractions`, `GetShortestPath`

### Examples

```
#Create a temporary folder, or anywhere you want:
SA_AnalysisDir=file.path(tempdir(),'MACPETtest')
dir.create(SA_AnalysisDir)#where you will save the results
#give directory of the BAM file:
S2_PairedEndBAMpath=system.file('extdata', 'SampleChIAPETData.bam', package = 'MACPET')

#give prefix name:
SA_prefix='MACPET'

#parallel backhead can be created using the BiocParallel package
#parallel backhead can be created using the BiocParallel package
#requireNamespace('BiocParallel')
#snow <- BiocParallel::SnowParam(workers = 4, type = 'SOCK', progressBar=FALSE)
```

```
#BiocParallel::register(snow, default=TRUE)

#-run for the whole binding site analysis:
MACPETUlt(SA_AnalysisDir=SA_AnalysisDir,
          SA_stages=c(2:3),
          SA_prefix=SA_prefix,
          S2_PairedEndBAMpath=S2_PairedEndBAMpath,
          S2_image=TRUE,
          S2_BlackList=TRUE,
          S3_image=TRUE)

#load results:
SelfObject=paste(SA_prefix, '_pselfData', sep='')
load(file.path(SA_AnalysisDir, 'S2_results', SelfObject))
SelfObject=get(SelfObject)
class(SelfObject) # see methods for this class

IntraObject=paste(SA_prefix, '_pintraData', sep='')
load(file.path(SA_AnalysisDir, 'S2_results', IntraObject))
IntraObject=get(IntraObject)
class(IntraObject) # see methods for this class

InterObject=paste(SA_prefix, '_pinterData', sep='')
load(file.path(SA_AnalysisDir, 'S2_results', InterObject))
InterObject=get(InterObject)
class(InterObject) # see methods for this class

SelfFitObject=paste(SA_prefix, '_psfitData', sep='')
load(file.path(SA_AnalysisDir, 'S3_results', SelfFitObject))
SelfFitObject=get(SelfFitObject)
class(SelfFitObject) # see methods for this class

#-----delete test directory:
unlink(SA_AnalysisDir,recursive=TRUE)
```

---

MACPET\_GenomeMapData.rda

*Genomic interactions from ChIA-PET data*

---

## Description

Genomic Interactions data from ESR1 ChIA-PET subset data on human MCF-7.

## Format

rda object of [GenomeMap](#) class.

**Details**

MACPET\_GenomeMapData is produced by the [MACPETUlt](#) function at Stage 4 and it contains all the genomic interactions between the peaks in the data.

**Author(s)**

**Main data creators** Yijun Ruan, GIS, 2012-05-24

**MACPET\_GenomeMapData.rda creator** Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**See Also**

[GenomeMap](#)

---

MACPET\_pinterData.rda *Inter-chromosomal PETs from ChIA-PET data*

---

**Description**

Inter-chromosomal PETs data from ESR1 ChIA-PET subset data on human MCF-7.

**Format**

rda object of [PInter](#) class.

**Details**

MACPET\_pinterData is produced by the [MACPETUlt](#) function at Stage 2 and it contains the Inter-chromosomal PETs of the sample data.

**Author(s)**

**Main data creators** Yijun Ruan, GIS, 2012-05-24

**MACPET\_pinterData.rda creator** Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**See Also**

[SampleChIAPETData.bam](#), [PInter](#)



---

MACPET\_pintraData.rda *Intra-chromosomal PETs from ChIA-PET data*

---

**Description**

Intra-chromosomal PETs data from ESR1 ChIA-PET subset data on human MCF-7.

**Format**

rda object of [PIntra](#) class.

**Details**

MACPET\_pintraData is produced by the [MACPETUlt](#) function at Stage 2 and it contains the Intra-chromosomal PETs of the sample data.

**Author(s)**

**Main data creators** Yijun Ruan, GIS, 2012-05-24

**MACPET\_pintraData.rda creator** Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**See Also**

[SampleChIAPETData.bam](#), [PIntra](#)

---

MACPET\_pselfData.rda *Self-ligated PETs from ChIA-PET data*

---

**Description**

Self-ligated PETs data from ESR1 ChIA-PET subset data on human MCF-7.

**Format**

rda object of [PSelf](#) class.

**Details**

MACPET\_pselfData is produced by the [MACPETUlt](#) function at Stage 2 and it contains the Self-ligated PETs of the sample data.

**Author(s)**

**Main data creators** Yijun Ruan, GIS, 2012-05-24

**MACPET\_pselfData.rda creator** Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**See Also**

[SampleChIAPETData.bam](#), [PSelf](#)

---

MACPET\_psfिटData.rda *Self-ligated PETs from ChIA-PET data*

---

**Description**

Self-ligated PETs data from ESR1 ChIA-PET subset data on human MCF-7.

**Format**

rda object of [PSFit](#) class.

**Details**

MACPET\_psfिटData is produced by the [MACPETUlt](#) function at Stage 3 and it contains the self-ligated PETs of the sample data after calling for candidate peaks.

**Author(s)**

**Main data creators** Yijun Ruan, GIS, 2012-05-24

**MACPET\_psfिटData.rda creator** Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**See Also**

[SampleChIAPETData.bam](#), [PSFit](#)

---

PeaksToGRanges *Convert peaks to GRanges object*

---

**Description**

PeaksToGRanges converts peaks of an object of [PSFit](#) class to [GRanges](#) object.

**Usage**

```
PeaksToGRanges(object, ...)

## Default S3 method:
PeaksToGRanges(object, ...)

## S3 method for class 'PSFit'
PeaksToGRanges(object, threshold = NULL, ...)

## S4 method for signature 'PSFit'
PeaksToGRanges(object, threshold = NULL, ...)
```

**Arguments**

object	An object of class <a href="#">PSFit</a> .
...	Further arguments to be passed to PeaksToGRanges (not used).
threshold	A numeric with the FDR cut-off threshold used to take a subset of significant peaks. If threshold=NULL then all the peaks are returned.

**Details**

PeaksToGRanges converts peak information into a [GRanges](#) object. Each row in the [GRanges](#) object represents a peak with 'CIQ.Up.start' and 'CIQ.Down.end' as start and end coordinates, respectively (see [PSFit](#)) Metadata will also include information for the total PETs, the p-value and the FDR of each peak.

**Value**

For [PSFit](#) class, a [GRanges](#) object created by the estimated peak information including metadata columns for the total PETs, the p-value and the FDR of each peak.

**Author(s)**

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**References**

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

**See Also**

[PSFit](#), [PeaksToNarrowPeak](#)

**Examples**

```
#load Self-ligated data: (class=PSFit)
load(system.file('extdata', 'MACPET_psfिटData.rda', package = 'MACPET'))
class(MACPET_psfिटData)
PeaksToGRanges(object=MACPET_psfिटData, threshold=1e-5)
```

---

PeaksToNarrowPeak      *Convert Peaks to narrowPeak (BED) object.*

---

### Description

PeaksToNarrowPeak converts peaks of an object of `PSFit` class to narrowPeak object. The object is saved in a user specified directory and can be used in the MANGO or MICC algorithms for interaction analysis. Alternatively, the user can run stage 4 at [MACPETUI.t](#).

### Usage

```
PeaksToNarrowPeak(object, ...)

## Default S3 method:
PeaksToNarrowPeak(object, ...)

## S3 method for class 'PSFit'
PeaksToNarrowPeak(object, threshold = NULL, savedir,
  file.out, ...)

## S4 method for signature 'PSFit'
PeaksToNarrowPeak(object, threshold = NULL, savedir,
  file.out, ...)
```

### Arguments

object	An object of class <code>PSFit</code> .
...	Further arguments to be passed to PeaksToNarrowPeak (not used).
threshold	A numeric with the FDR cut-off threshold used to take a subset of significant peaks. If threshold=NULL then all the peaks are returned.
savedir	A string with the directory to save the output file.
file.out	A string with the name of the output to be saved to savedir.

### Details

Each Peak in the narrowPeak object is represented by an interval starting from the 'CIQ.Up.start' estimated variable to its 'CIQ.Down.end' (see `PSFit`). Close Peaks in genomic distance are NOT merged by the PeaksToNarrowPeak function. However the user can specify a distance window for merging in the MANGO or MICC algorithms. Note also that MANGO and MICC find a self-ligated cut-off by itself which is usually very different than that found by MACPET. We suggest that the user overwrites MANGOS's or MICC's cut-off with that of MACPET.

### Value

A narrowPeak object named after the value of file.out and saved in the savedir.

**Author(s)**

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**References**

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

**See Also**

[PSFit](#)

**Examples**

```
#Create a temporary folder, or anywhere you want:
savedir=file.path(tempdir(), 'MACPETtest')
dir.create(savedir)#where you will save the results
file.out='MACPET_peaks.narrowPeak'

#load Self-ligated data: (class=PSFit)
load(system.file('extdata', 'MACPET_psfiteData.rda', package = 'MACPET'))
class(MACPET_psfiteData)
PeaksToNarrowPeak(object=MACPET_psfiteData, threshold=1e-5, file.out=file.out, savedir=savedir)

#----delete test directory:
unlink(savedir, recursive=TRUE)
```

---

PInter-class

*PInter S4 Class*

---

**Description**

[PInter](#) class in a S4 class which inherits from the [GInteractions](#) class and it contains Inter-chromosomal data.

**Details**

[PInter](#) class is created by the [MACPETU1t](#) function at Stage 2.

**Author(s)**

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**References**

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

**See Also**

[AnalysisStatistics](#), [plot](#), [summary](#), [MACPETUlt](#)

---

PIntra-class

*PIntra S4 Class*

---

**Description**

[PIntra](#) class in a S4 class which inherits from the [GInteractions](#) class and it contains Intra-chromosomal data.

**Details**

[PIntra](#) class is created by the [MACPETUlt](#) function at Stage 2.

**Author(s)**

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**References**

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

**See Also**

[AnalysisStatistics](#), [plot](#), [summary](#), [MACPETUlt](#)

---

plot

*plot methods for MACPET classes*

---

**Description**

Different plot methods for the classes in the [MACPET](#) package.

**Usage**

```
## S3 method for class 'PInter'
plot(x, ...)
```

```
## S3 method for class 'PIntra'
plot(x, ...)
```

```
## S3 method for class 'PSelf'
plot(x, ...)
```

```
## S3 method for class 'PSFit'
plot(x, kind, RegIndex = NULL, threshold = NULL, ...)

## S3 method for class 'GenomeMap'
plot(x, Type, threshold = NULL, ...)
```

### Arguments

x	An object of correct class used to create different plots.
...	further arguments to be passed in the plot functions.
kind	A string with one of the following arguments. Note that if a region visualization is plotted, the vertical lines represent peak-summits. PETcounts For a bar-plot of the PET-counts in each chromosome. RegionCounts For a bar-plot for the region counts in each chromosome. PeakCounts For a bar-plot for the Peak-counts in each chromosome. RegionPETs For a ggplot for a visualization of the PETs in a region. RegionTags For a ggplot for a visualization of the tags in a region. The tags are classified by stream (upper/lower) PeakPETs For a ggplot for a visualization of the PETs in a region. The PETs are classified by the peak they belong to. PeakTags For a ggplot for a visualization of the tags in a region. The tags are classified by the peak they belong to. SigPETCounts For a bar-plot with the significant PET-counts in each chromosome. SigRegionCounts For a bar-plot with the significant region-counts in each chromosome. SigPeakCounts For a bar-plot with the significant peak-counts in each chromosome.
RegIndex	an integer indicating which region to plot (1 means the biggest in terms of total PETs.)
threshold	The FDR cut-off when plotting the total significant peaks/interactions for each chromosome in the data.
Type	A string with one of the following values: 'heatmap', 'network-random', 'network-circle', 'network-...'. Each one shows the relations between the chromosomes based on their interactions.

### Value

For the [PInter](#) class: A network plot. Each node is a chromosome with size proportional to the total PETs of the corresponding chromosome. Edges connect chromosomes which have common PETs, where the thickness of an edge is proportional on the total number of PETs connecting the two chromosomes.

For the [PIntra](#) class: A bar-plot. Each bar represents the total number of Intra-chromosomal PETs for each chromosome in the data.

For the [PSelf](#) class: A bar-plot. Each bar represents the total number of Self-ligated PETs for each chromosome in the data.

For the [PSFit](#) class: Different plots depending on the kind argument.

For the [GenomeMap](#) class: A heatmap plot or different kinds of network plots showing the relations between different chromosomes based on the interactions between them.

### Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

### References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Complete pipeline for ChIA-PET*. To be published.

### See Also

[PSelf](#), [PSFit](#) [PInter](#), [PIntra](#), [GenomeMap](#)

### Examples

```
#load Inter-chromosomal data:
load(system.file('extdata', 'MACPET_pinterData.rda', package = 'MACPET'))
class(MACPET_pinterData)
requireNamespace('igraph')
plot(MACPET_pinterData)

#load Intra-chromosomal data:
load(system.file('extdata', 'MACPET_pintraData.rda', package = 'MACPET'))
class(MACPET_pintraData)
requireNamespace('ggplot2')
plot(MACPET_pintraData)

#load Self-ligated data:
load(system.file('extdata', 'MACPET_pselfData.rda', package = 'MACPET'))
class(MACPET_pselfData)
requireNamespace('ggplot2')
plot(MACPET_pselfData)

#load Self-ligated data:
load(system.file('extdata', 'MACPET_psfitData.rda', package = 'MACPET'))
class(MACPET_psfitData)
requireNamespace('ggplot2')
plot(MACPET_psfitData, kind='PETcounts')
plot(MACPET_psfitData, kind='PeakCounts')
plot(MACPET_psfitData, kind='PeakPETS', RegIndex=1)
plot(MACPET_psfitData, kind='PeakTags', RegIndex=1)

#load Interactions data:
load(system.file('extdata', 'MACPET_GenomeMapData.rda', package = 'MACPET'))
class(MACPET_GenomeMapData)
```



```
requireNamespace('igraph')
requireNamespace('reshape2')
plot(MACPET_GenomeMapData, Type='network-circle')
```

---

PSelf-class

*PSelf S4 Class*

---

## Description

[PSelf](#) class in a S4 class which inherits from the [GInteractions](#) class and it contains Self-ligated PETs from ChIA-PET experiment. Furthermore it also contains the following in the [metadata](#) field:

`Self_info` A data.frame with the count statistics for the total PETs in each chromosome.

`SLmean` The mean size of the PETs in the data.

`MaxSize` Maximum size of self-ligated PETs.

`MinSize` Minimum size of self-ligated PETs.

## Details

[PSelf](#) class is created by the [MACPETUlt](#) function at Stage 2.

## Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

## References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

## See Also

[AnalysisStatistics](#), [plot](#), [summary](#), [MACPETUlt](#), [ConvertToPSelf](#)

PSFit-class

*PSFit S4 Class***Description**

**PSFit** class in a S4 class which inherits from the **GInteractions** class and it contains Self-ligated PETs from ChIA-PET experiment and information about the genome of the data. Furthermore it also contains the following in the **metadata** field:

**Self\_info** Counts statistics for the total PETs, total regions and total Peaks in each chromosome.

**SLmean** The mean size of the PETs in the data.

**MaxSize** Maximum size of self-ligated PETs.

**MinSize** Minimum size of self-ligated PETs.

**Classification.Info** A matrix with information for the Data-row ID, region ID and Peak ID (0 represent noise) of each peak in the data.

**Peaks.Info** Information for each peak found by the peak-calling algorithm:

**Chrom** The chromosome which the peak belongs to.

**Region** The region which the peak belongs to.

**Peak** The peak ID (a region might have more than one peaks).

**Pets** Total PETs in the peak.

**Peak.Summit** Summit of the peak.

**Up.Summit** Summit of the left-stream PETs.

**Down.Summit** Summit of the right-stream PETs.

**CIQ.Up.start** Start of the 95 Quantile confidence interval for the left-stream PETs.

**CIQ.Up.end** End of the 95 Quantile confidence interval for the left-stream PETs.

**CIQ.Up.size** Size of the 95 Quantile confidence interval for the left-stream PETs.

**CIQ.Down.start** Start of the 95 Quantile confidence interval for the right-stream PETs.

**CIQ.Down.end** End of the 95 Quantile confidence interval for the right-stream PETs.

**CIQ.Down.size** Size of the 95 Quantile confidence interval for the right-stream PETs.

**CIQ.Peak.size** Size of the Peak based on the interval (CIQ.Up.start,CIQ.Down.end).

**sdx** The standard deviation of the upstream PETs.

**lambdax** The skewness of the upstream PETs.

**sdyl** The standard deviation of the downstream PETs.

**lambday** The skewness of the downstream PETs.

**lambdaUp** The expected number of PETs in the left-stream Peak region by random chance.

**FoldEnrichUp** Fold enrichment for the left-stream Peak region.

**p.valueUp** p-value for the left-stream Peak region.

**lambdaDown** The expected number of PETs in the right-stream Peak region by random chance.

**FoldEnrichDown** Fold enrichment for the right-stream Peak region.

**p.valueDown** p-value for the right-stream Peak region.

**p.value** p-value for the Peak ( $p.valueUp * p.valueDown$ ).

**FDRUp** FDR correction for the left-stream Peak region.

**FDRDown** FDR correction for the right-stream Peak region.

**FDR** FDR correction for the Peak.

**Details**

`PSFit` class is created by the `MACPETUlt` function at Stage 3.

**Author(s)**

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**References**

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

**See Also**

[AnalysisStatistics,plot](#), [summary](#), [MACPETUlt](#), [exportPeaks](#), [PeaksToGRanges](#), [TagsToGInteractions](#), [PeaksToNarrowPeak](#)

---

SampleChIAPETData.bam *Subset sample of ChIA-PET data*

---

**Description**

A subset of ChIA-PET data:

**Target:** ESR1

**Biosample summary:** Homo sapiens MCF-7

**GEO:** GSM970212

**Format**

A BAM file.

**Author(s)**

Yijun Ruan, GIS, 2012-05-24 (main data creators)

**Source**

<https://www.encodeproject.org/experiments/ENCSR000BZZ/>

**References**

Consortium EP (2012) *An integrated encyclopedia of DNA elements in the human genome..* Nature, 489(7414), pp. 57–74. <http://dx.doi.org/10.1038/nature11247>.

**See Also**

[MACPET\\_pinterData.rda](#), [MACPET\\_pintraData.rda](#), [MACPET\\_pselfData.rda](#), [MACPET\\_psfidData.rda](#), [SampleChIAPETDataRead\\_1.bam](#), [SampleChIAPETDataRead\\_2.bam](#)

---

SampleChIAPETDataRead\_1.bam

*First reads from a subset of ChIA-PET data in*  
[SampleChIAPETData.bam](#)

---

### Description

First reads from a subset of ChIA-PET data in [SampleChIAPETData.bam](#):

**Target:** ESR1

**Biosample summary:** Homo sapiens MCF-7

**GEO:** GSM970212

### Format

A BAM file.

### Author(s)

Yijun Ruan, GIS, 2012-05-24 (main data creators)

### Source

<https://www.encodeproject.org/experiments/ENCSR000BZZ/>

### References

Consortium EP (2012) *An integrated encyclopedia of DNA elements in the human genome..* Nature, 489(7414), pp. 57–74. <http://dx.doi.org/10.1038/nature11247>.

### See Also

[SampleChIAPETData.bam](#), [ConvertToPE\\_BAM](#)

---

SampleChIAPETDataRead\_2.bam

*Second reads from a subset of ChIA-PET data in*  
[SampleChIAPETData.bam](#)

---

### Description

Second reads from a subset of ChIA-PET data in [SampleChIAPETData.bam](#):

**Target:** ESR1

**Biosample summary:** Homo sapiens MCF-7

**GEO:** GSM970212

**Format**

A BAM file.

**Author(s)**

Yijun Ruan, GIS, 2012-05-24 (main data creators)

**Source**

<https://www.encodeproject.org/experiments/ENCSR000BZZ/>

**References**

Consortium EP (2012) *An integrated encyclopedia of DNA elements in the human genome.* Nature, 489(7414), pp. 57–74. <http://dx.doi.org/10.1038/nature11247>.

**See Also**

[SampleChIAPETData.bam](#), [ConvertToPE\\_BAM](#)

---

summary

*summary methods for the MACPET classes.*

---

**Description**

Different summary methods for the classes in the [MACPET](#) package.

**Usage**

```
## S3 method for class 'PSelf'  
summary(object, ...)  
  
## S4 method for signature 'PSelf'  
summary(object, ...)  
  
## S3 method for class 'PSFit'  
summary(object, threshold = NULL, ...)  
  
## S4 method for signature 'PSFit'  
summary(object, threshold = NULL, ...)  
  
## S3 method for class 'PIntra'  
summary(object, heatmap = FALSE, ...)  
  
## S4 method for signature 'PIntra'  
summary(object, heatmap = FALSE, ...)
```

```
## S3 method for class 'PInter'
summary(object, heatmap = FALSE, ...)

## S4 method for signature 'PInter'
summary(object, heatmap = FALSE, ...)

## S3 method for class 'GenomeMap'
summary(object, threshold = NULL, ...)

## S4 method for signature 'GenomeMap'
summary(object, threshold = NULL, ...)
```

### Arguments

object	An object of correct class used to create different summaries.
...	Further arguments to be passed to the summary function.
threshold	A numeric representing the FDR cut-off or summarizing significant peaks (or interactions), if NULL the summary is based on all the peaks found.
heatmap	TRUE or FALSE indicating whether the user wants to plot a heat-map plot for the Intra/Inter-chromosomal PET counts within chromosomes or between different chromosomes.

### Value

A summary of the object and a heat-map plot depending on the class of the input.

### Author(s)

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

### References

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

### See Also

[PSelf](#), [PSFit](#), [PInter](#), [PIntra](#), [GenomeMap](#)

### Examples

```
#load Self-ligated data: (class=PSelf)
load(system.file('extdata', 'MACPET_pselfData.rda', package = 'MACPET'))
class(MACPET_pselfData)
summary(MACPET_pselfData)

#load Self-ligated data: (class=PSFit)
load(system.file('extdata', 'MACPET_psfitData.rda', package = 'MACPET'))
class(MACPET_psfitData)
summary(MACPET_psfitData)
```

```

summary(MACPET_psfData,threshold=1e-5)

#load Intra-chromosomal data: (class=PIIntra)
load(system.file('extdata', 'MACPET_pintraData.rda', package = 'MACPET'))
class(MACPET_pintraData)
summary(MACPET_pintraData)
requireNamespace('ggplot2')
requireNamespace('reshape2')
summary(MACPET_pintraData,heatmap=TRUE)#sample data, not good heatmap plot.

#load Inter-chromosomal data: (class=PIInter)
load(system.file('extdata', 'MACPET_pinterData.rda', package = 'MACPET'))
class(MACPET_pinterData)
summary(MACPET_pinterData)
requireNamespace('ggplot2')
requireNamespace('reshape2')
summary(MACPET_pinterData,heatmap=TRUE)#sample data, not good heatmap plot.

#load Interaction data: (class=GenomeMap)
load(system.file('extdata', 'MACPET_GenomeMapData.rda', package = 'MACPET'))
class(MACPET_GenomeMapData)
summary(MACPET_GenomeMapData)

```

---

TagsToGInteractions     *Convert PETs to GInteractions object*

---

## Description

TagsToGInteractions converts the PETs of an object of [PSFit](#) class to [GInteractions](#) object.

## Usage

```

TagsToGInteractions(object, ...)

## Default S3 method:
TagsToGInteractions(object, ...)

## S3 method for class 'PSFit'
TagsToGInteractions(object, threshold = NULL, ...)

## S4 method for signature 'PSFit'
TagsToGInteractions(object, threshold = NULL, ...)

```

## Arguments

object	An object of class <a href="#">PSFit</a> .
...	(not used).
threshold	A numeric for the FDR threshold used to take a subset of significant peaks/binding sites. If threshold=NULL then all the peaks are returned.

**Value**

For `PSFit` class: A `GInteractions` object containing PETs from all the peaks found in the data (removing noisy and insignificant PETs). Furthermore, it also includes information about the binding sites which can be accessed via the `metadata` function.

**Author(s)**

Ioannis Vardaxis, <ioannis.vardaxis@ntnu.no>

**References**

Vardaxis I, Drabløs F, Rye M and Lindqvist BH (2018). *MACPET: Model-based Analysis for ChIA-PET*. To be published.

**See Also**

[PSFit](#)

**Examples**

```
#load Self-ligated data: (class=PSFit)
load(system.file('extdata', 'MACPET_psfiteData.rda', package = 'MACPET'))
class(MACPET_psfiteData)
object=TagsToGInteractions(object=MACPET_psfiteData,threshold=1e-5)
object
S4Vectors::metadata(object)$Peaks.Info #peak/binding site information
```



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